IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant :	Charles D. Jones	)	
Serial No.:	331,042	)	Group Art Unit: 121
Filed :	December 16, 1981	)	Examiner: R. Schwartz
For :	ANTIESTROGENIC AND ANTI-ANDROGENIC BENZOTHIOPHENE	)	
Docket No.:	X-5526A	)	

11-30-82

DECLARATION UNDER 37 C.F.R. 1.132

James H. Clark, Ph.D. declares as follows:

I am a professor in the department of cell biology, Baylor College of Medicine, Houston, Texas. I earned my Ph.D. degree in the field of endocrinology at Purdue University in 1968, and have been engaged in research and education in physiology, more particularly in the physiology of steroids and hormones, ever since. I was a post-doctoral fellow at the University of Illinois from 1968 until 1970, an assistant and later associate professor in the department of biological science at Purdue University from 1970 through 1973, and came to my present department at Baylor in 1973. I attained the rank of full professor in 1977.

Since 1973, I have been or still am a member of 15 national and international scientific committees. I have been or presently am a member of the editorial board of eight scientific journals which publish in my field, such as the Journal of Steroid Biochemistry, Endocrine Research Communications, The Journal of Receptor Research and Endocrinology.

Since 1970, my research has been in part supported by 12 grants from the U.S. Public Health Service-National Institutes of Health or the American Cancer Society. I have presented about 94 invited talks and seminars at various symposia, scientific

meetings and research organizations, at which I reported my research. I am an editor of four books on hormones and anti-hormones, and the mechanism of their action at the cell level, and am an author of two books, of which the more recent is Female Sex Steroids: Receptors and Function, Monographs In Endocrinology, Springer-Verlag, Heidelberg (1979), with E. J. Peck, Jr. I am an author of about 83 published articles, and of about 37 chapters and symposia volumes, as well as of about 13 publications now in press.

I have seen tissues from the uteri of animals used in the experiments of Larry J. Black of Eli Lilly and Company, in which immature rats were dosed with 6-hydroxy-2-(4-hydroxyphenyl)-3-[4-(2-piperidinoethoxy)benzoyl]benzo[b]thiophene (compound I) and 6-hydroxy-2-(4-hydroxyphenyl)-3-[4-(2-pyrrolidinoethoxy)-benzoyl]benzo[b]thiophene (compound II). Technicians working under my supervision have prepared sections and slides of such tissues, and have made photomicrographs of the slides, some of which are presented as exhibits with this document.

The photographs presented here, which are representative of the tissues which I have seen from animals treated with the different test compounds and control treatments, are of course not all of the slides which my staff has made and which I have studied. No attempt is made here to do more than to present slides which illustrate the effects on uterine tissue which I have observed. The enclosed photographs, which I have chosen for this purpose, are as follows.

Exhibit 1 - Untreated control, ovariectomized rat, test 930.

Exhibit 2 - Untreated control, immature rat, test 917.

Exhibit 3 - Estradiol, 0.1 microgram per day for 8 days,

ovariectomized rat, test 930.

Exhibit 4 - Estradiol, 0.1 microgram per day for 3 days, immature rat, test 917.

Exhibit 5 - Compound I, 1000 micrograms per day for 3 days, immature rat, test 917.

Exhibit 6 - Compound II, 1000 micrograms per day for 3 days, immature rat, test 917.

Exhibit 7 - Compound I, 1000 micrograms per day for 3 days, ovariectomized rat, test 934.

Exhibit 8 - Compound II, 1000 micrograms per day for 3 days, ovariectomized rat, test 934.

Exhibit 9 - Estradiol for 3 days, then estradiol plus 1000 micrograms per day of compound I for 5 days, ovariectomized rat, test 930.

Exhibit 10- Estradiol for 3 days, then estradiol plus 1000 micrograms per day of compound II for 5 days, ovariectomized rat, test 930.

The first manner in which compounds I and II are compared is with regard to their relative estrogenic activities. Exhibit 2, an untreated immature rat uterus, is compared with Exhibits 5 and 6, the uteri of immature rats treated with compound I and compound II, respectively. Compound II, as is graphically seen in Example 6, stimulates epithelial cell hypertrophy rather extensively. The stimulation caused by compound II is similar to that seen with a physiological dose of estradiol, Exhibit 4. In contrast, compound I (Exhibit 5) shows little if any stimulation of the epithelial cells, and hence its estrogenicity is near zero.

Exhibits 7 and 8 show the effects of treatment of ovariectomized rats with compound I and compound II, at high doses for three days in each case. The untreated control tissue is Exhibit 1, and an estradiol-treated tissue is seen in Exhibit 3. In this model, compound I exhibits more estrogenicity than it does in the immature rat, but the difference between compounds I and II is readily visible in the photographs. Compound II clearly causes more hypertrophy of the epithelial cells of the uterus than does compound I. In this model as well, the effect of compound II approaches that of a physiological dose of estradiol, Exhibit 3.

Exhibits 9 and 10 show the effect on uterine tissue of first creating a strong estrogenic response with estradiol, and then administering the combination of estradiol and one of the test compounds. The difference between the two treatments is quite striking. The administration of compound I with estradiol, seen in Exhibit 9, has greatly decreased epithelial cell hypertrophy. In contrast, administration of compound II with estradiol (Exhibit 10), has not affected the epithelial cell hypertrophy, but has left the cells in substantially the same condition as the controls (Exhibit 3) treated with estradiol alone.

Therefore, the major difference between compound I and compound II is the very low to nonexistent estrogenicity of compound I when compared to compound II.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or

imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

James H. Clark

Oct 27/82
Date